Thermal characterization of the anthocyanins from black soybean (*Glycine max* L.) exposed to thermogravimetry

Dan Wang, Yue Ma, Chao Zhang, Xiaoyan Zhao*

Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Science, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China) and Key Laboratory of Urban Agriculture (North), Ministry of Agriculture, Beijing 100097, PR China

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ABSTRACT

One anthocyanin compound, cyanin-3-glucoside, was isolated and identified by high performance liquid chromatography tandem mass spectrometry (HPLC_MS/MS) from seed coat of black soybean variety, Heizi to assess its thermal characterization in vitro. The thermal characterization of anthocyanin was studied by thermogravimetry (TG) analysis. The results from the TG curves showed the decomposition of anthocyanin occurred in three stages. Glucose cleaved from the cyanin-3-glucoside occurred in the first stage. Degradation of cyanin and small amounts of cyanin-3-glucoside appeared mainly in the second stage. The sugar in the anthocyanin was decomposed in the third stage. Thermodynamic analysis was applied by free dynamic model. TG-isothermal experiments for the anthocyanins from black soybean seed coats confirmed the correct of the thermodynamic analysis.

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1. Introduction

Black soybean (*Glycine max* L.) accumulates high amounts of anthocyanins, which base with cyanidin, delphinidin, petunidin, and pelargonidin, almost exclusively as 3-*O*-gluscosides in the seed coat (Choung et al., 2001; Kovinich, Saleem, Arnason, & Miki, 2010; Lee et al., 2009). However, the anthocyanin compositions are diverse among different black soybean varieties (Choung et al., 2001; Kuroda & Wada, 1933; Yoshikura & Hamaguchi, 1969). Anthocyanins in black soybean seed coat have antioxidant activities, α-glucosidase inhibition, regulation of adhesion molecules, protection from ischemia, reperfusion heart injury, stimulation wound healing in fibroblasts, and prevention inflammation in endothelial cells (Inagaki, Morimura, Shigematsu, Kida, & Akutagawa, 2005; Kim et al., 2006; Matsui et al., 2001; Nizamutdinova et al., 2009). In addition, they had in vitro antioxidant activity in human low density lipoprotein (Astadi, Astuti, Santoso, & Nugraheni, 2009).

Anthocyanins are unstable compounds which can induce the chemical transformation and affect the performance and biological activity of anthocyanin-containing foods. It was postulated that anthocyanins degraded by two pathways. One is the loss of glycosyl moieties to form aglycone, followed by the formation of a more

unstable α-diketone, and then aldehydes and benzoic acid derivatives are formed (Sadilova, Stintzing, & Carle, 2006). Another pathway was postulated that the pseudobase was formed first, followed by the formation of chalcones and coumarin glycosides, and the chalcones would undergo a further degradation. When anthocyanins are exposed to different conditions, they exhibit different degradation mechanisms which induce different degradation route (Zhang, Sun, Hu, & Liao, 2010). However, the degradation mechanism of anthocyanins from black soybean seed coat has not been reported yet.

Many foods are thermally processed prior to consumption and this process can greatly influence the anthocyanin contents in the final products (Giusti & Wrolstad, 2003; Patras, Brunton, Donnell, & Tiwari, 2010). Thermodynamic analysis can give a deep insight into the anthocyanin conversion. Many anthocyanin degradations have been reported to follow the first-order kinetic model based on prediction (Gradinaru, Biliaderis, Kallithraka, Kefalas, & Garcia-Viguera, 2003; Wang & Xu, 2007; Yang, Han, Gu, Fan, & Chen, 2008). However, for simple reactions the evaluation of reaction degree with the *n*-th order model is possible. For complex reactions the function of reaction degree is complicated and not generally known. In such cases the *n*-th order algorithm produces unreasonable kinetic data. However, model free kinetics is based on the theory that reaction degree and the activation energy are constant for a certain value of conversion (iso-conversional method). With model free kinetics, accurate evaluations of complex and simple

* Corresponding author. Tel./fax: +86 10 51503053.
  E-mail addresses: xiaoyanzhao001@yahoo.com.cn, zhaoxiaoyan@nercv.org (X. Zhao).

reactions can be performed. Therefore, the reaction degree of anthocyanins from black soybean exposed to thermogravimetric apparatus was evaluated with model free kinetics in this research.

In this study, anthocyanin was identified by high performance liquid chromatography tandem mass spectrometry (LC_MS/MS) from black soybean variety, Heizi seed coat. Furthermore, the thermal characterization and degradation mechanism of anthocyanin were studied by thermogravimetric (TG) analysis applied by free dynamic model. TG-isothermal experiments for the anthocyanins from black soybean seed coats were used to confirm that the thermodynamic analysis was suitable. Results would be useful for the application of anthocyanins from black soybean seed coat as colorants or antioxidants in foods.

2. Materials and methods

2.1. Chemicals

Ethanol anhydrous, methanol, acetontrile were purchased from Dima Technologies (Beijing, China). Formic acid ($\geq$98%) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Standard of cyanin-3-glucoside was obtained from Polyphenols Laboratories AS (Sandnes, Norway). These solvents/chemicals used were chromatography grade. Syringe filter units 0.22 μm were supplied by Hercules (Beijing, China). Distilled water was used.

2.2. Extraction and purification of anthocyanins

Black soybean variety, Heizi was supplied by Beijing Academy of Agriculture and Forestry Sciences (Beijing 2011, China). Hand-peeled seed coats were extracted twice with 60% ethanol (pH = 3.0). Anthocyanins extraction conditions were: the volume ratio of black soybean seed coat and extracted solution was 1:15; temperature, 50 °C; extraction time, 1 h. Resultant extraction solution was concentrated with a rotary evaporator (BŰCHI R-215, Flawil, Switzerland) then filtered by a 0.22 μm syringe filter. The extracts were applied on an Amberlite XAD-7 column (60 cm × 1.6 cm, Sigma, Santa clara, USA). After washing the column with 10-fold column volume H$_2$O contained 0.5 mL/100 mL TFA, the extracted anthocyanins were eluted by methanol containing 0.5 mL/100 mL TFA. The elution was evaporated.

Then 5 mL concentrated isolated methanol pigments were fractioned by Sephadex LH-20 chromatograph (60 cm × 1.6 cm, GE, Fairfield, USA) using H$_2$O (0.5 mL/100 mL TFA)-MeOH (7:3) as eluent. The flow rate was 1.0 mL/min. Fractions were collected by a fraction auto-collector (GE, Fairfield, USA). The fractions were evaporated on a rotary evaporator to remove methanol to facilitate the removal of water remaining in the sample. The evaporation temperature was less than 45 °C. The removal of water was carried out on a freeze drier (LGJ-10, Beijing Songyuan Huaxing Technology Developing Co., Beijing, China). The homogeneity of individual fraction was checked by analytical HPLC-DAD. Then they resolved in distilled water containing 0.5 mL/100 mL formic acid and filtered through a 0.22 μm syringe filter for HPLC_MS/MS analysis and TG experiment.

2.3. HPLC_MS/MS analysis

The HPLC_MS/MS analysis of purified anthocyanins were performed by an Agilent 1200 series liquid chromatograph containing an autosampler coupled to a 6300 series ion trap mass spectrometer (Agilent, Santa Clara, USA). 3 μL of the sample was injected onto an analytical scale Zorbax SB-AO column (particle size, 1.8 μm; 100 × 3.0 mm, Agilent, Santa Clara, USA) maintained at 25 °C. The elution mode was a linear acetonitrile gradient (10%–60%, 30 min)

containing 0.5% formic acid at a flow rate of 0.3 mL/min for HPLC_MS/MS analysis.

DAD detection at 280 and 520 nm were performed. Anthocyanins were detected using ion trap in the positive ion mode. Used MS parameters were as follows: nebulizing pressure, 30 psi; source temperature, 110 °C; desolvation temperature, 350 °C; desolvation gas flow, 11 L/min nitrogen; scan ranger, m/z 100–1500; smart parameter setting, compound stability, 20%; trap drive level, 100%. Identification of anthocyanidin compound was based on the retention time and LC/MS m/z values with reference to cyanin-3-glucoside standard.

2.4. Thermal characterization of anthocyanins from black soybean seed coat

Thermodynamic characterization of the sample was measured by a Mettler Toledo thermogravimetry (Zürich, Switzerland), over a temperature interval of 30–900 °C at heating rate of 5, 10, 15 °C/min, respectively. The thermograph was analyzed by the stare software (version 9.30). The thermodynamic parameter and characterization of the sample was studied. Especially, the degree of conversion (α) of the sample with time and its relationship with temperature were studied. To confirm the accuracy of dynamic analysis by model free kinetics, the TG-isothermal curves were obtained over 10 min at temperatures of 170, 200, 210, 250 °C, which the temperatures of joints between the first and second stage of anthocyanin decomposition, as observed in the TG dynamic profiles.

3. Results and discussion

3.1. Purification and identification of anthocyanins from black soybean seed coat

Anthocyanins were extracted from black soybean seed coats with 60% ethanol. There was only one peak in HPLC chromatogram of the crude extract detected at 520 nm, while several peaks at 280 nm (data not shown), which indicating that resultant solution was a mixture containing one kind of anthocyanin, phenolic acids, sugars, proteins, and other flavonoids. Amberlite XAD-7 was chosen to separate the compounds above. Sugars and proteins have no affinity for the resin and can be washed away by water with 0.5 mL/100 mL TFA. The compounds are continuously isolated using Shephdex LH-20, which remove the phenolic acids and other flavonoids. Anthocyanins, which are the target compounds, were collected and identified by high-performance liquid chromatography (HPLC). 6 mg purified anthocyanin was obtained from 100 g black soybean seed coat.

HPLC chromatogram of the anthocyanin fraction at 520 and 280 nm as a detection wavelength was shown in Fig. 1A. There was only one peak with a retention time of 8.1 min in Fig. 1A. Furthermore, mass spectrometry was used to detect the molecular mass and to identify the structure. A single prominent protonated molecular ion peak was found at m/z 449 [M + H]$^+$ in the MS spectrum, indicating only one compound in peak (Fig. 1B). Further results (Fig. 1C) were found by using MS/MS. One fragment ion (m/z 287) was found in MS/MS spectrum, produced through losing one group with mass of 787–625 = 162 from parent ion, indicating sugar moiety as the lost fragment. Finally, peak 1 was identified as cyanin-3-glucoside according to their mass spectra, previous research and the reference of standard (Islam, Yoshimoto, & Terahara, 2002; Truong et al., 2010). UV/visible spectra of this peak performed by a diode array detector were characterized between 200 and 800 nm (data not shown), which confirming that the compound was cyanin-3-glucoside. According to the results above, there was only one
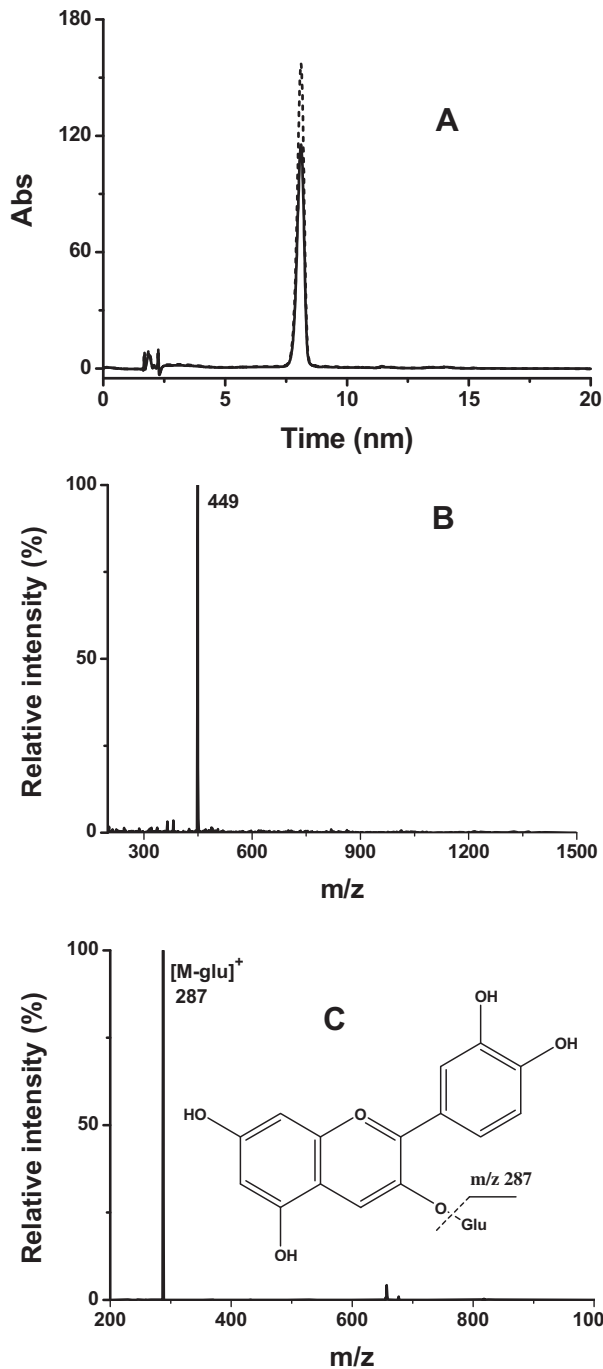
Fig. 1. HPLC chromatogram, MS and MS/MS spectra of anthocyanins from black soybean seed coat. (A) HPLC chromatogram (— 520 nm, - - - 280 nm); (B) MS spectra; (C) MS/MS spectra.
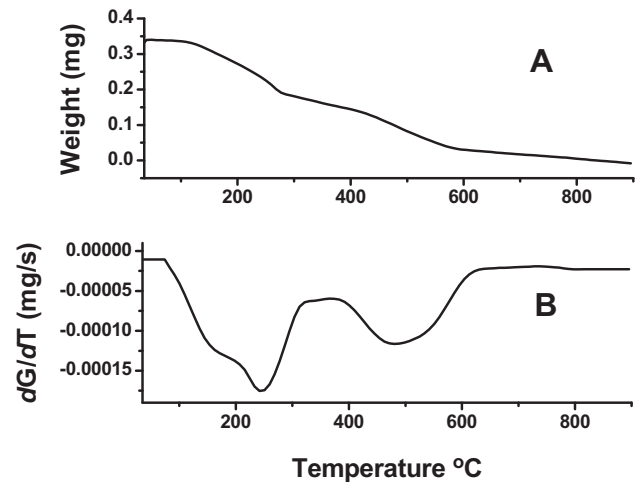

Fig. 2. Thermal stability of anthocyanin from black soybean seed coats under nitrogen atmosphere. (A) TG curves; (B) DTG curves.

product (Giusti & Wrolstad, 2003). Thermodynamic analysis can give a deeper insight into anthocyanin conversion. The TG curve (heating rate is 10 °C/min) and its corresponding DTG curve (differential coefficient of TG curve) were displayed in Fig. 2. The TG and DTG curves showed the decomposition of anthocyanin

kind of anthocyanin in this black soybean variety, which was less than other varieties (Choung, 2008). However, these findings are in accordance with previous researches, who found that cyanindin 3-glucoside was the major anthocyanin in black soybeans (Xu & Chang, 2008).

3.2. Thermal characterization of anthocyanins from black soybean seed coat

Many foods are thermally processed prior to consumption and this process can greatly influence anthocyanin content in the final
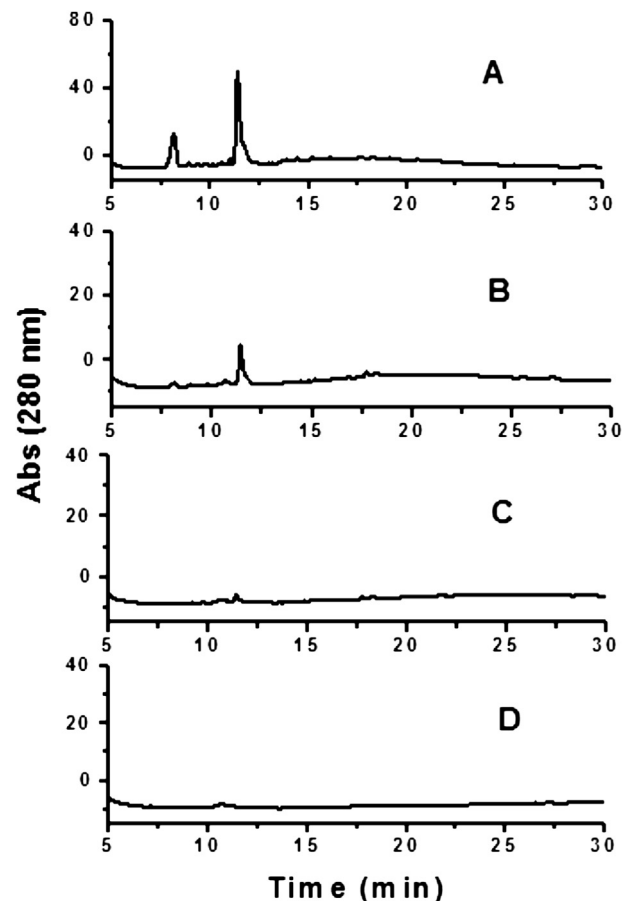

Fig. 3. HPLC chromatograms of anthocyanins after thermal treatments at heating rate of 10 °C/min. (A) Thermal treatment from 30 to 200 °C; (B) Thermal treatment from 30 to 250 °C; (C) Thermal treatment from 30 to 300 °C; (D) Thermal treatment from 30 to 390 °C.
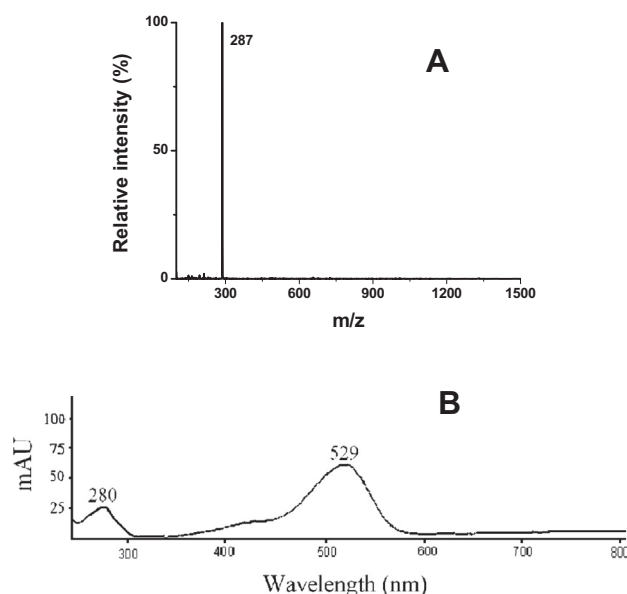
Fig. 4. MS and UV−vis spectra of cyanindin. (A) MS spectra; (B) UV−vis spectra.

occurring in three stages. The first and second stage were not separated completely, and the characterization of anthocyanin mainly changed in the first two decomposition stages, therefore, it was observed especially at 30−200, 250, 300, 390 °C, which were the typical temperatures between the first two decomposition stages, as observed in the TG dynamic profiles. HPLC chromatogram of the anthocyanin after thermal treatment at above temperatures with 280 nm as a detection wavelength was shown in Fig. 3. Prior to the thermal treatment, the typical pigment pattern of black soybean coat was found as cyanin-3-glucoside (Fig. 1A). After being heated to 200 °C, the amount of cyanin-3-glucoside was decreased to 13%, and an additional peak with a retention time of 11.8 min appeared (Fig. 3A). These findings are in accordance with Xu et al., who found boiling for 120 min and steaming for 100 min caused decrease of monomeric anthocyanin content in black soybean. The weight loss were 93% and 90.7%, respectively (Xu & Chang, 2008). Furthermore, it was observed that cyanidin 3-glucoside in black rice resulted in a loss of 74.2% during the cooking process using a rice cooker (Hiemori, Koh, & Mitchell, 2009). Comparing the data from the results and literature, the mass loss were different, since the different treatment conditions and raw materials. Cyanin-3-glucoside had shorter retention time than the new compound, indicating that cyanin-3-glucoside had stronger polarity. Due to its lower polarity and its higher absorption maximum of 529 nm compared to cyanin-3-glucoside, this compound was assigned to cyanin, which was confirmed by its specific molecular ion of m/z 287 (Fig. 4). It can be concluded that glucose cleaved from the cyanin-3-glucoside occurred and produced cyanin mainly in the first stage. After heating to 250 °C, the amount of both cyanin-3-

glucoside and cyanidin were decreased, especially, there is almost no cyanin-3-glucoside (Fig. 3B). After heating to 300 and 390 °C, anthocyanin degradated completely and nothing could be observed (Fig. 3C and D). The results could be related to a decomposition process with degradation of cyanin and small amounts of cyanin-3-glucoside mainly appeared in the second stage. However, there was a big mass loss after being heated to 390 °C. According to the stoichiometry for decomposition reaction, where the mass loss in the third stage was 37%, which corresponds to the sugar in the cyanin-3-glucoside. It proved that the sugar was decomposed in the third stage. The results were in agreement with thermal decomposition of sugars in rutin (Costa, Filho, Nascimeno, & Macêdo, 2002). It was proved that anthocyanin from this black soybean variety degraded by the loss of glycosyl moieties to form aglycone after thermal treatment.

Thermal characterizations of many anthocyanins have been evaluated by *n*-th order model (Gradinaru et al., 2003; Wang & Xu, 2007; Yang et al., 2008). However, for simple reactions the evaluation of reaction degree with the *n*-th order model is possible. For complex reactions, the function of reaction degree is complicated and not generally known. In such cases the *n*-th order algorithm produces unreasonable kinetic data. According to the TG results, the thermal degradation of anthocyanin from black soybean seed coat included continuous complex reactions. With model free kinetics, accurate evaluations could be performed. Model free kinetics are based on the theory that reaction degree and the activation energy are constant for a certain value of conversion (isoconversional method), which were observed in the TG and DTG dynamic profiles.

After the activation energy was calculated according to Vyazovkin theory, which changed with the conversion (Vyazovkin, 2008), the degree of anthocyanin conversion could be predicted and controlled manually over a wide range of temperatures and time by the thermodynamics calculation. The relationships between the degree of conversion and time or temperature were shown in Table 1. It represented the percentage of the reacted samples versus time at a given isothermal temperature or a given time. It is clear that the degree of conversion increased as the temperature increased. The thermodynamics calculation showed that the $t_{1/2}$ values for anthocyanin from black soybean seed coat degradation were 157.58, 105.4, 93.33, 2.71, 1.57, 1.31, 0.69 and 0.37 h at 25, 50, 100, 170, 200, 210, 250 and 300 °C, respectively, which varied from 157.58 to 0.37 h (Table 1). These results have a great relevance to food processing procedures, such as blanching, pasteurization, baking, frying and roasting. These processing methods require high temperatures and do not involve any change of pH, which will be useful for black soybean processing.

TG-isothermal curves were obtained over 10 min at temperatures of 170, 210, and 250 °C, which are temperatures of joints between the first and second stage of anthocyanin decomposition, as observed in the TG dynamic profiles (Fig. 5). The results showed that the 10% conversion times of anthocyanin were 5.3, 2.53, and 1.27 min at 170, 210, and 250 °C, respectively (Fig. 5). Furthermore,

Table 1
Time (min) for certain conversion degree of the anthocyanins from black soybean seed coats at different temperatures.

Degree of conversion (%)	Temperature (°C)							
	25	50	100	170	200	210	250	300
10	1128.4 ± 1.8	317.2 ± 1.2	44.2 ± 1.8	5.8 ± 0.1	3.9 ± 0.1	3.0 ± 0.1	1.3 ± 0.07	0.9 ± 0.06
20	5189.8 ± 2.7	2107.9 ± 1.6	191.8 ± 0.3	17.5 ± 0.5	11.2 ± 0.1	8.2 ± 0.08	3.4 ± 0.06	3.2 ± 0.04
30	5207.7 ± 2.5	5581.9 ± 1.1	4559.2 ± 0.4	87.2 ± 0.4	19.3 ± 0.1	14.1 ± 0.4	5.5 ± 0.1	5.5 ± 0.1
50	9454.7 ± 2.0	6323.9 ± 1.6	5599.7 ± 1.2	162.4 ± 0.6	93.9 ± 1.1	78.6 ± 0.7	41.5 ± 1.0	22.1 ± 0.4

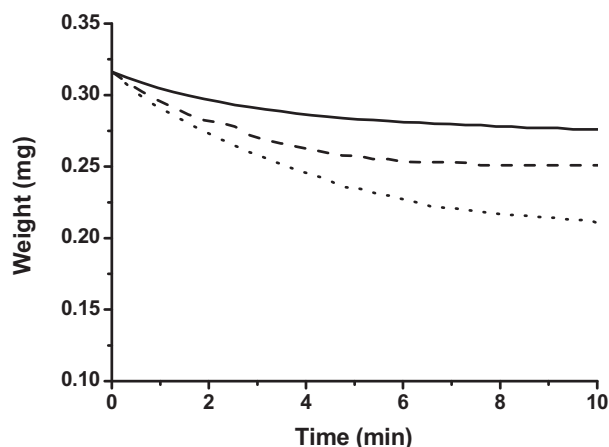Each value is the mean of three replications ± standard deviation.

Fig. 5. TG-isothermal curves for the anthocyanins from black soybean seed coat (—170 °C, - - - 210 °C, · · · 250 °C).

the 20% conversion times of anthocyanin were 7.6 and 3.48 min at 210 and 250 °C, and degrees of conversion were 30% in 5.7 min at 250 °C (Fig. 5). The time for the percent of the sample reacted at a given isothermal temperature was in good agreement with the time predicted by thermodynamics calculation, which confirmed the accuracy of dynamic analysis by model free kinetics. The thermodynamics calculation could provide valuable information to evaluate the thermal stability of anthocyanins from black soybean seed coat when applied in extraction and thermal processing.

4. Conclusions

In this study, the anthocyanin identified from black soybean contained three decomposition stages exposed to thermogravimetry. Glucose cleaved from the cyanin-3-glucoside occurred in the first stage. Degradation of cyanin and small amounts of cyanin-3-glucoside appeared mainly in the second stage. The sugar in the anthocyanin was decomposed in the third stage. The relationships between the degree of conversion and time or temperature of anthocyanin from black soybean seed coat predicted by the thermodynamics calculation using free dynamic model was correct, which was also confirmed by TG-isothermal experiments. The elucidation of thermal characterization of anthocyanin will be helpful for clarity of the structure change of black soybean anthocyanins during heating processing.

Acknowledgment

References

Astadi, I. R., Astuti, M., Santoso, U., & Nugraheni, P. S. (2009). In vitro antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). *Food Chemistry, 112*, 659–663.

Choung, M. G. (2008). Optimal HPLC condition for simultaneous determination of anthocyanins in black soybean seed coats. *Korean Journal of Crop Science, 53*, 359–368.

Choung, M., Baek, I. Y., Kang, S. T., Han, W. Y., Shin, D. C., Moon, H. P., et al. (2001). Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* (L.) Merr.). *Journal of Agricultural and Food Chemistry, 49*, 5848–5851.

Costa, E. M., Filho, J. M. B., Nascimeno, T. G., & Macêdo, R. O. (2002). Thermal characterization of the quercetin and rutin flavonoids. *Thermochimica Acta, 392–393*, 79–84.

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal, 14*, 217–225.

Gradinaru, G., Biliaderis, C. G., Kallithraka, S., Kefalas, P., & Garcia-Viguera, C. (2003). Thermal stability of *Hibiscus sabdariffa* L. anthocyanins in solution and in solid state: effects of copigmentation and glass transition. *Food Chemistry, 83*, 423–436.

Hiemori, M., Koh, E., & Mitchell, A. E. (2009). Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *japonica* var. SBR). *Journal of Agricultural and Food Chemistry, 57*, 1908–1914.

Inagaki, S., Morimura, S., Shigematsu, T., Kida, K., & Akutagawa, H. (2005). Apoptosis induction by vinegar produced from boiled extract of black soybeans in human monoblastic leukemia U937 cells: difference in sensitivity to cell toxicity compared to normal lymphocytes. *Food Science and Technology Research, 3*, 311–317.

Islam, M. S., Yoshimoto, M., & Terahara, N. (2002). Anthocyanin compositions in sweetpotato (*Ipomoea batatas* L.) leaves. *Bioscience Biotechnology Biochemistry, 66*, 2483–2486.

Kim, H. J., Tsoy, I., Park, J. M., Chung, J. I., Shin, S. C., & Chang, K. C. (2006). Anthocyanins from soybean seed coat inhibit the expression of TNF-a-induced genes associated with ischemia/reperfusion in endothelial cells by NF-κB-dependent pathway and reduce rat myocardial damages incurred by ischemia and reperfusion in vivo. *FEBS Letters., 580*, 1391–1397.

Kovinich, N., Saleem, A., Arnason, J. T., & Miki, B. (2010). Functional characterization of a UDP-glucose: flavonoid 3-O-glucosyltransferase from the seed coat of black soybean (*Glycine max* (L.) Merr.). *Phytochemistry, 71*, 1253–1263.

Kuroda, C., & Wada, M. (1933). The colouring matter of kuro-mame. In *Proceedings of the Imperial Academy* (Vol. 9; pp. 17).

Lee, J. H., Kang, N. S., Shin, S. O., Shin, S. H., Lim, S. G., Suh, D. Y., et al. (2009). Characterization of anthocyanins in the black soybean (*Glycine max* L.) by HPLC-DAD-ESI/MS analysis. *Food Chemistry, 112*, 226–231.

Matsui, T., Ueda, T., Oki, T., Sugita, K., Terahara, N., & Matsumoto, K. (2001). a-Glucosidase inhibitory action of natural acylated anthocyanins. 2. a-Glucosidase inhibition by isolated acylated anthocyanins. *Journal of Agricultural and Food Chemistry, 49*, 1952–1956.

Nizamutdinova, I. T., Kim, Y. M., Chung, J., Shin, S. C., Jeong, Y. K., Seo, H. G., et al. (2009). Anthocyanins from black soybean seed coats stimulate wound healing in fibroblasts and keratinocytes and prevent inflammation in endothelial cells. *Food and Chemical Toxicology, 47*, 2806–2812.

Patras, A., Brunton, N. P., Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology, 21*, 3–11.

Sadilova, E., Stintzing, C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science, 71*, C504–C512.

Truong, V. D., Deighton, N., Thompson, R. T., Mcfeeters, R. F., Dean, L. O., Pecota, K. V., et al. (2010). Characterization of anthocyanins and anthocyanidins in purple-fleshed sweetpotatoes by HPLC-DAD/ESI-MS/MS. *Journal of Agricultural and Food Chemistry, 58*, 404–410.

Vyazovkin, S. (2008). Isoconversional kinetics. *Handbook of Thermal Analysis and Calorimetry, 5*, 503–538.

Wang, W. D., & Xu, S. Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentration. *Journal of Food Engineer, 82*, 271–275.

Xu, B., & Chang, S. K. C. (2008). Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry, 56*, 7165–7175.

Yang, Z., Han, Y., Gu, Z., Fan, G., & Chen, Z. (2008). Thermal degradation kinetics of aqueous anthocyanins and visual color of purple corn (*Zea mays* L.) cob. *Innovative Food Science Emerging Technology, 9*, 341–345.

Yoshikura, M., & Hamaguchi, Y. (1969). Anthocyanins of the black soybean. *Eiyo To Shokuryo, 22*, 367.

Zhang, Y., Sun, J., Hu, X., & Liao, X. (2010). Spectral alteration and degradation of cyanidin-3-glucoside exposed to pulsed electric field. *Journal of Agricultural and Food Chemistry, 58*, 3524–3531.